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Usher Syndrome in the Samaritans: Strengths and Limitations of Using Inbred Isolated Populations to Identify Genes Causing Recessive Disorders

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ABSTRACT We have previously reported significant linkage between markers on 11q13.5 and Usher syndrome type 1 (USH1B) in a large Samaritan kindred. USH1B is an autosomal recessive disease characterized by profound congenital sensorineural deafness, vestibular dysfunction and progressive visual loss. A unique haplotype found only in all USH1B carriers and affected individuals implied that the disease-causing mutation probably entered the community from a single founder. Screening for mutations in a gene called GARP, which was mapped to the same genetic interval as USH1B, revealed a base substitution in the coding region of the gene, in a homozygous state in all affected individuals. This base substitution, which results in an arginine to tryptophane change, is not found in control individuals and occurs at an amino acid residue that is conserved across species, including mouse, gorilla, chimpanzee and macaque. This study emphasizes the strength of using an isolated inbred population for efficient identification of the primary linkage and for narrowing the disease interval, but also demonstrates its limitations in distinguishing between mutations causing the disease and those representing unique and private polymorphisms. Am J Phys Anthropol 104:193–200, 1997.

Large consanguineous inbred families with multiple affected offspring in several sibships provide a powerful tool for the localization of disease genes by linkage analysis. Only within the last 2 years, at least eight different genetic loci, responsible for recessive non-syndromic hearing loss, were localized to chromosomal regions. All of them were identified, indeed, in consanguineous kindreds from geographically and ethnically isolated populations: two different loci in Tunisian families (Guilford et al., 1994a,b), three from India (Fukushima et al., 1995a,b; Jain et al., 1995), two from the Middle East, Lebanon and Northern Israel (Chaib et al., 1995; Baldwin et al., 1995) and one large family from Bali (Friedman et al., 1995). Another example for a successful identification of significant linkage in an inbred kindred between a recessive disease gene (Usher Syndrome) and markers on the long arm of chromosome 11 is our recent study on the Samaritans (Bonné-Tamir et al., 1994). Upon

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localization of the gene, efforts were directed to fine mapping and to haplotype analysis of the region (Seroussi et al., 1994; Korostishevsky et al., 1994). An Usher carrier haplotype was found to be different from all other haplotypes and was found only in obligate carriers and affected individuals. The uniqueness of the haplotype implied that the Usher mutation probably entered the Samaritan community from a single founder and that all Samaritans with Usher carry the same mutation which can be traced to this common ancestor. In this study we present results of continued attempts to map a possible candidate gene to the Usher linkage region and of our endeavors to detect a putative mutation within this gene in the Samaritan Usher patients. This gene, called GARP, was previously characterized by Ollendorff et al. (1992, 1994) and has characteristics, including similarity to genes involved in development of sensory organs in Drosophila, which support its candidacy as an USHER type 1 gene.

THE SAMARITANS

The Samaritan community in the Middle East has only about 590 members and is the solitary heir of a continuous religious and cultural tradition going back to an early stage in biblical history. Once a nation of several thousand people, they have gradually become a small sect that lives in two localities in the same geographical area in which they have remained for a period of more than 2,000 years. Extensive demographic and genetic investigations of the Samaritan community have been carried out since the 1960s (Bonné 1963, 1966a,b). The Samaritans exhibit a unique profile with extreme frequencies in almost all genetic traits which differ significantly from those found in Jewish and in non-Jewish populations in the region. They demonstrate a high preference for intra-lineage marriages, with 84% of matings between either first or second cousins. Their inbreeding coefficient is the highest recorded for any human population (Bonné-Tamir, 1980). The genetic constitution of the present-day population derives from only 45 founders. Detailed pedigrees documenting the last 13 generations allow calculation of the probabilities of origin of the genes and the total number of descendants from each founder (Cazes and Bonné-Tamir, 1984). There are five major patrilineages in the community that are not equally distributed between the two localities. Usher type 1 (congenital hearing loss) was found to affect particular members of one lineage (Fig. 1).

THE USHER SYNDROMES

The Usher (USH) syndromes are a group of genotypically distinct diseases which share several phenotypic characteristics. Named after Charles Usher, a British ophthalmologist, they are characterized by autosomal recessive inheritance of both congenital bilateral sensorineural deafness and retinitis pigmentosa (RP). Variation in symptoms among affected families led investigators to propose several distinct Usher phenotypes known as USH type 1, USH type 2 and USH type 3. Because of the phenotypic differences, genetic heterogeneity was hypothesized in 1977 (Davenport and Omenn, 1977) and recently verified by linkage studies. The gene causing USH2 was first assigned by Kimberling et al. (1990) and Lewis et al. (1990) to the long arm of chromosome 1. However, not all USH2 families mapped to this region. Families diagnosed as USH1 showed linkage to three different loci: chromosome 11p (USH1A) (Smith et al., 1992), chromosome 11q (USH1B) (Kimberling et al., 1992) and chromosome 14q (USH1C) (Kaplan et al., 1991). Recently a fourth location (USH1D) (Larget-Piet et al., 1995) has been suggested on basis of excluding the previous three loci. USH3 was assigned to chromosome 3q in families from Finland (Sankila et al., 1995). Thus, linkage studies demonstrated locus heterogeneity and established the existence of at least seven different loci responsible for the defect.

METHODS

PCR amplification

Genomic DNA was extracted from blood of 112 individuals by previously described methods (Sambrook et al., 1989) and typed for microsatellite markers, including a biallelic polymorphism within the 3′UTR of the GARP gene, on chromosome 11q. Typing of

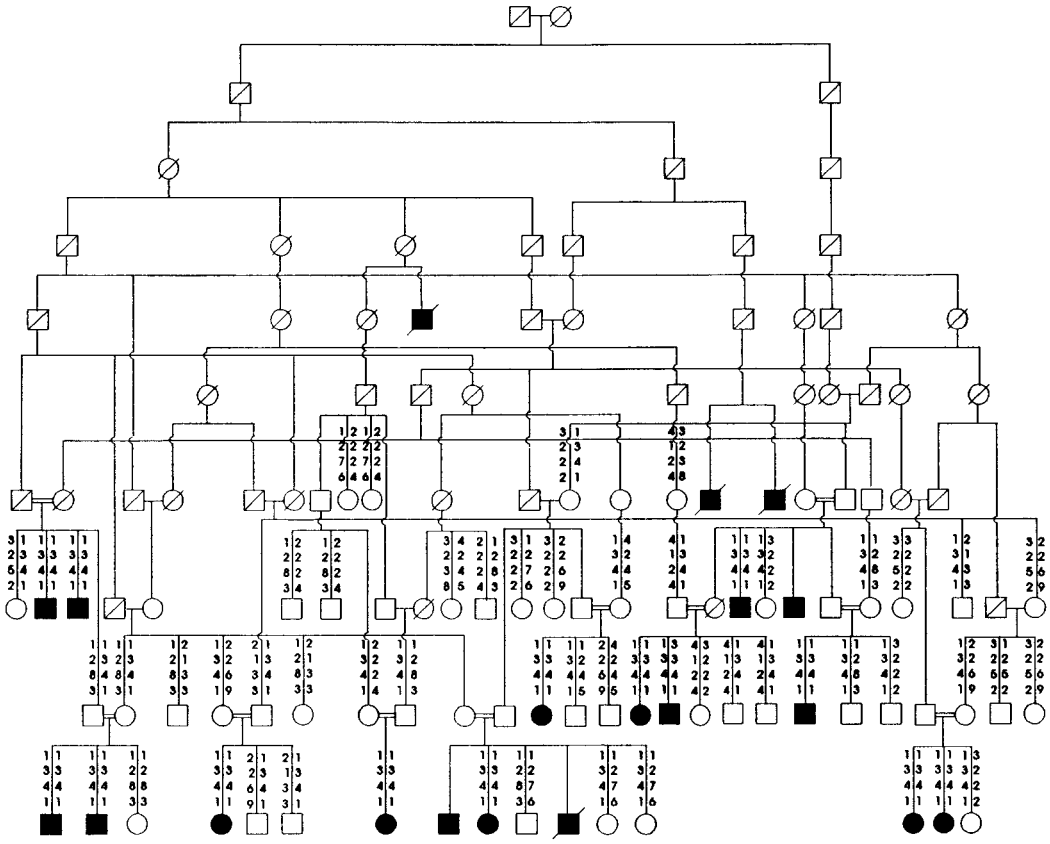


Fig. 1. Samaritan Usher kindred. Affected individuals are represented by the solid symbols and unaffected individuals are represented by open symbols. The genotyping data for four closely linked polymorphisms are shown above each symbol (from top to bottom: D11S916, GARP, D11S527, D11S533).

markers was performed using PCR amplification techniques. Specific oligonucleotides primers were used for each marker.

SSCP analysis

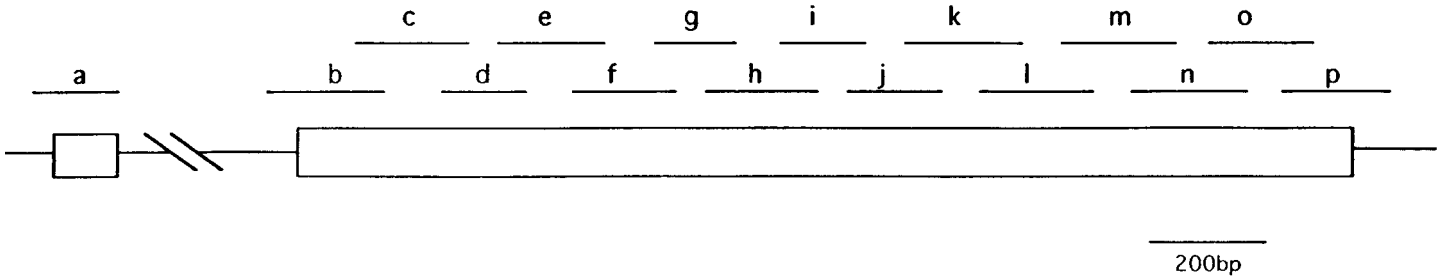
PCR primers which spanned the entire coding region of the GARP gene in overlapping segments of approximately 200 bp or less were designed (Fig. 2). Single strand conformation polymorphism (SSCP) analysis was used to analyze each of the PCR products, generated by these primers using DNA from affected individuals and obligate carriers from the Samaritan kindred.

Sequencing

PCR products showing a band shift by SSCP analysis were gel purified and used as templates for fluorescent cycle sequencing.

RESULTS

Previous studies using cytogenetic mapping methods localized the GARP gene to chromosome 11q13.5–11q14 region. Since the USH1B locus is also located in the region, GARP was considered a candidate gene for this disorder based on cytogenetic map position. In an attempt to either exclude GARP as the cause of USH1B or to more precisely place it within the linkage region, we searched for a genetic polymorphism within the gene using SSCP analysis. Using primers within the 3′UTR of the gene we identified a biallelic polymorphism. Two point linkage analysis was performed with this marker and other polymorphic markers using the CHLC linkage server. Two point analysis placed GARP between markers



Fragment	Primer Sequence	cDNA nucleotide position	Fragment	Primer Sequence	cDNA nucleotide position
a	TTGCTTTGGAGACAGATGAAC CTTACAGGGCACTTTGTCTTG	First exon	i	GATTGAGCTCATCCCCGAC GTGTCTCCAGGGCATTGTG	1072-1226
b	CACTTATGCCAGCTGTCTCC AGCTGATCTCATTGGTGCTC	3' First intron 353	j	GCCTGATGCTCCTTGACTTA GTTCCCCTGCAGGTTGAG	1185-1354
c	CTTCTACACGGCACTTCGTC GTACAGGCTGTTCCCAGACA	304-505	k	CCATACACCTTTGCCAATCT AAGGTCCAGCTCAGTCAGTG	1301-1513
d	ACTGCGCTGAGTGCTGGT GTGAGGCGAGTCAGACT	431-588	l	TGAGATAGAGCTGCTCAGGG CAAGATTGAGCCGCTTGAG	1453-1658
e	AGCCTGCATACCCTCTCACT GAAGTCGGAGATGCAGGTGA	542-739	m	ATGGTCCTGCAGGTGGAC TGGATTCCCCTGCAGGTAGA	1604-1810
f	CATCTCAACCTCTCCAGGAA TCGGGGAAATGGAGCAGTTT	695-873	n	CGAAACAACAGCTTCAGCCT GTGGCTCAGGGACACCTC	1724-1930
g	TCCAGCTCACCTGGCTTGA GTGGATGCCCTTGCTGTC	822-967	o	GATCTGCCGCTTCAGCTC TGTTGGTTAAACTTCTGCCG	1888-2070
h	CTTGTCCAACAACCTCATCC GAGGTCAGGTGCTCAAGAAA	907-1113	p	GAGGTGTCCCTGAGCCAC CCTAGAGTGTCTCCCGGCTT	1913-2105

Fig. 2. Schematic drawing showing the GARP gene and the position of the PCR products designed to cover the entire coding region of this gene. The primer sequences for each PCR product are shown.

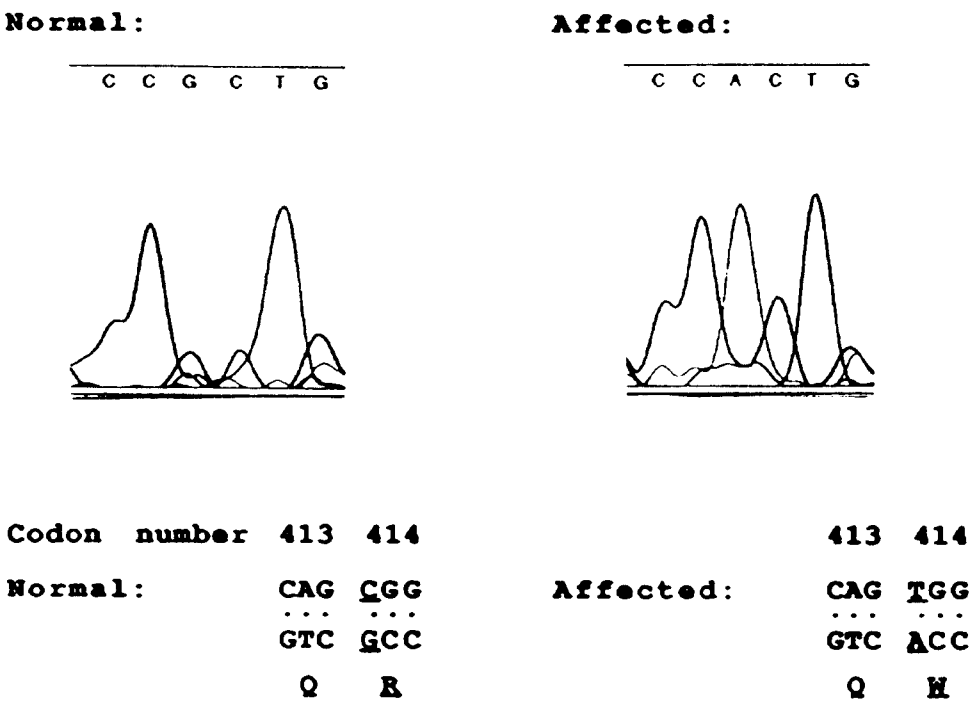


Fig. 3. Fluorescent sequencing waveforms showing a base substitution resulting in an arginine to tryptophan change at amino acid residue 414 (Arg414Trp). The sequence shown is the sense (template) strand (G to A change). Sequencing of the antisense strand confirmed this result.

D11S916 and D11S918. There were no recombinants with D11S533, the most closely linked marker to USH1B in the Samaritan kindred. The GARP 3′UTR polymorphism was then used to genotype all individuals within the Samaritan Usher kindred. This polymorphism proved to be very informative and segregated without recombination with the disease phenotype in this kindred (Fig. 1). A homozygous shift was identified in all affected individuals in one of the PCR products spanning the coding region of the GARP gene. This shift was also present in the heterozygous state in all obligate carriers. Sequencing of this PCR product revealed an arginine to tryptophan substitution at codon 414 (Arg414Trp) (Fig. 3). In order to determine if this amino acid substitution was likely to be a protein polymorphism, over 200 control individuals were screened using SSCP analysis. This substitution was not found in any control individuals, including more than 50 Samaritans. In addition, human PCR primers flanking codon 414 were used to amplify this region of the GARP gene from the following species: mouse, gorilla, chimpanzee and macaque. Sequencing of this region from each of these species showed that the arginine residue was conserved across all species, even though, in the case of the mouse, an alternative arginine codon is used (Adato et al., 1996). In order to further evaluate whether or not mutations in the GARP gene cause USHER syndrome, we screened 45 unrelated non-Samaritan Usher patients for mutations using the GARP SSCP assay. These patients all carried the diagnosis of Usher syndrome based on the presence of both deafness and retinal degeneration. One sequence change was identified in four individuals which proved to be an alanine to threonine substitution at codon 407 (Ala407THR) (Adato et al., 1996). This substitution was found in a homozygous state

in two individuals and in a heterozygous state in two other individuals. This alanine sequence is conserved across species. Evaluation of 100 control individuals identified this base substitution in the heterozygous state in a single individual, who could be a carrier of USH1B.

DISCUSSION

The GARP gene was first identified and mapped to the region of chromosome 11q13.5-11q14 by Ollendorff et al. (1992). Results of the current study place this gene between flanking markers D11S916 and D11S527 in the region of USH1B. Little is known about the function of the GARP protein. What is known suggests that mutation within the GARP gene could cause Usher syndrome. GARP codes for a putative transmembrane protein consisting of 662 amino acids. The major portion of the protein consists of 20 leucine-rich repeat (LRR) domains (Ollendorff et al., 1994). Due to the LRR domains, the GARP protein (Garpin) has structural similarities to other proteins, including chaoptin and connectin, which are involved in the development of sensory organs and the nervous system in Drosophila (Ollendorff et al., 1992). The two amino acid substitutions identified in this study both occur within an LRR domain and may disrupt the tertiary structure of the Garpin protein (Mindrinos et al., 1994; Whitman et al., 1994; Bent et al., 1994). A biallelic polymorphism with the 3′UTR of the GARP gene and a potential disease causing amino acid substitution within this gene were shown in this study to fully segregate with the Usher disease phenotype in a large inbred Samaritan kindred. Segregation with the disease phenotype is an important criteria for any candidate gene proposed to cause a specific inherited disorder. An additional criteria for a disease causing mutation is that the sequence alteration result in a plausible protein mutation. The base substitution reported here results in an arginine (a basic amino acid) to tryptophan (an aromatic amino acid) change in an amino acid residue shown to be conserved across diverse species. In addition we failed to find this change in 200 control individuals. However, these data *do not prove* that the GARP gene causes USH1B. Additional evidence of the involvement of GARP in Usher syndrome would be the identification of additional sequence changes within the gene in other Usher patients. To investigate this possibility, we screened DNA for GARP mutations from 45 additional Usher patients available to us. In four patients, another sequence change, analanine to threonine substitution at codon 407 was identified: in a homozygous state in a Tunisian and a Moroccan Jew and in a heterozygous state in two North Americans. Recent studies (Weil et al., 1995) in non-Samaritan families suggested that another gene, a defective Myosin VIIA gene, is responsible for USH1B. Hence, in spite of the segregation of the GARP gene with the disease phenotype, the data *does not allow* us to distinguish between a mutation causing the disease and a private polymorphism which characterizes the Samaritans.

This study, thus, reveals both the strengths and the limitations of using inbred isolated groups to identify genes causing rare recessive disorders. Such populations are extremely useful for identifying the primary linkage and for narrowing the disease interval. However, once the interval has been narrowed to the smallest possible region, using all available meioses, any gene within the linkage region remains a candidate. Any base substitution found within any of the candidate genes can be argued to be a unique polymorphism found in an isolated founder population. Studying additional unrelated patients is essential for determining the role of GARP in Usher syndrome.

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When we first became interested, over three decades ago, in the demography of the Samaritans, the studies of Derek F. Roberts

of the DINKA and Northeast Tanganyika tribes provided us with significant insights and suggestions. Acquaintance with the Samaritans themselves led Derek to direct involvement in the search of the influence of the Samaritan's extreme inbred mating system on their genetic attributes, their reproductive histories and the extent of variability in quantitative traits, such as digital dermatoglyphics (see Roberts and Bonné (1973), Bonné-Tamir, Roberts and Coope (1982).

We express our sincere thanks and appreciation to Derek F. Roberts for his encouragement and interest which accompanied our continuous curiosity in the study of this unique group.

NOTE ADDED IN PROOF

Since the preparation of this paper, additional studies have identified the usher 1B mutation in the Samaritans, as a stop-coden in exon 40 of the myosin VII A gene (Adato et al., 1997).

LITERATURE CITED

Adato A, Kalinski H, Pel-Or Y, Korostishevsky M, and Bonné-Tamir B (1996) Usher syndrome in Jewish Morroccan families. Cell. Pharm. *3:*231–236.

Adato A, Weil D, Kalinski H, Pel-Or Y, Ayadi H, Petit C, Korostishevsky M, and Bonné-Tamir B (1997) Mutation profile of all 49 exons of the human myosin VII A gene, and haplotype analysis in usher 1B families from diverse origins. Am. J. Hum. Genet. (in press).

Baldwin CT, Farrer LA, Weiss S, De Stefano AL, Adair R, Franklyn B, Kidd KK, Korostishevsky M, and Bonné-Tamir B (1995) Linkage of congenital, recessive deafness (DFNB4) to chromosome 7q31 and evidence for genetic heterogeneity in the Middle Eastern Druze population. Hum. Mol. Genet. *4:*1637–1642.

Bent AF, Kunkel BN, Dahlbeck D, Brown KL, Schmidt R, Giraudat J, Leung J, and Staskawicz BJ (1994) RPS2 of arabidopsis thaliana: A leucine-rich repeat class of plant disease resistance genes. Science *265:* 1856–1860.

Bonné B (1963) The Samaritans: A demographic study. Hum. Biol. *35:*61–89.

Bonné B (1996a) Genes and phenotypes in the Samaritan isolate. Am. J. Phys. Anthropol. *24:*1–20.

Bonné B (1996b) Are there Hebrews left? Am. J. Phys. Anthropol. *24:*135–145.

Bonné-Tamir B (1980) The Samaritans: A living ancient isolate. In AW Eriksson et al. (eds.): Population Structure and Genetic Disorders. London: Academic, pp 27–41.

Bonné-Tamir B, Roberts DF, and Coope E (1982) Digital dermatoglyphics of the Samaritans. Hum. Hered. *32:*335–343.

Bonné-Tamir B, Korostishevsky M, Kalinsky H, Seroussi E, Beker R, Weiss S, and Godel V (1994) Genetic mapping of the gene for Usher syndrome: Linkage analysis in a large Samaritan kindred. Genomics *20:*36–42.

Cazes MH and Bonné-Tamir B (1984) Genetic evolution of the Samaritans. J. Biosoc. Sci. *16:*177–187.

Chaib H, Dodé C, Place C, Ayadi H, Loiselet J, Vincent C, and Petit C (1995) The chromosomal localization and cloning of human genes involved in sensorineural non-syndromic recessive deafness. Symposium on Molecular Biology of Hearing and Deafness, Bethesda, M.D. Abstract. p. 22.

Davenport SLH and Omenn GS (1977) The heterogeneity of Usher syndrome. Pub. 426. Amsterdam Excerpta Media Foundation. International Congress Series. Abstract *215:*87–88.

Friedman TB, Liang Y, Weber JL, Hinnant JT, Barber TD, Winata S, Arhya IN, and Asher JH Jr (1995) A gene for cogenital, recessive deafness DFNB3 maps to the pericentromeric region of chromosome 17. Nature Genet. *9:*86–91.

Fukushima K, Arabandi R, Srisailapathy CRS, Ni L, Chen A, O'Neill M, Van Camp G, Coucke P, Smith SD, Kenyon JB, Jain P, Wilcox ER, Zbar RIS, and Smith RJH (1995a) Consanguineous nuclear families used to identify a new locus for recessive non-syndromic hearing loss on 14q. Hum. Mol. Genet. *4:*1643–1648.

Fukushima K, Arabandi R, Srisailapathy CRS, Ni L, Wayne S, O'Neill ME, Van Camp G, Coucke P, Jain P, Wilcox ER, Smith SD, Kenyon JB, Zbar RIS, and Smith RJH (1995b) An autosomal recessive nonsyndromic form of sensorineural hearing loss maps to 3pDFNB6. Genome Res. *5:*305–308.

Guilford P, Arab SB, Blanchard S, Levilliers J, Weissenbach J, Belkahia A, and Petit C (1994a) A nonsyndromic form of neurosensory, recessive deafness maps to the pericentromeric region of chromosome 13q. Nature Genet. *6:*24–28.

Guilford P, Ayadi H, Blanchard S, Chaib H, Le Paslier D, Weissenbach J, Drira M, and Petit C (1994b) A human gene responsible for neurosensory, non-syndromic recessive deafness is a candidate homologue of the mouse sh-1 gene. Hum. Mol. Genet. *3:*989–993.

Jain, PK, Iukushima K, Deshmuk HD, Arabandi R, Thomas E, Kumar S, Lalwani AK, Ploplis B, Skarka H, Srisailpathy CRS, Wayne S, Zbar RIS, Verma IC, Smith RJH, Wilcox ER. A human recessive neurosensory nonsyndromic hearing impairment locus is a potential homologue of the murine deafness (dh) locus. Hum. Mol. Genet. *4:*2391–2394.

Kaplan J, Gerber S, Bonneau D, Rozet JM, Briard ML, Dufler JL, Munnich A, and Frezal J (1991) Probable location of Usher type I gene on chromosome 14q by linkage with D14S13 (MLJ14 probe). Cytogenet. Cell Genet. *58:*A27446.

Kimberling WJW, Weston MD, Moller C, Davenport SLH, Shugart YY, Priluck IA, Martini A, and Smith RJH (1990) Localization of Usher syndrome type II to chromosome 1q. Genomics *7:*245–249.

Kimberling WJ, Moller CG, Davenport S, Priluck IA, Beighton PH, Greenberg J, Reardon W, Weston MD, Kenyon JB, Grunkemeyer JA, Pieke Dahl S, Overbeck LD, Blackwood DJ, Brower AM, Hoover DM, Rowland P, and Smith RJH (1992) Linkage of Usher syndrome type I gene (USH1B) to the long arm of chromosome 11. Genomics *14:*988–994.

Korostishevsky M, Kalinsky H, Seroussi E, Weiss S, Vainder M, Sheffield VC, and Bonné-Tamir B (1994) Haplotype analysis of DNA microsatellites tightly linked to the locus of Usher syndrome type I on chromosome 11q. Am. J. Hum. Genet. *55:*A191.

Larget-Piet D, Gerber S, Rozet JM, Bonneav D, Mathieu M, Der Klaustian V, Munnich A, and Kaplan J (1995)

Evidence for a fourth locus responsible for Usher syndrome type I (USID). Am. J. Phys. Anthropol. *57:*A325. Abstract.

Lewis RA, Otterud B, Stauffer D, Lalouel JM, and Leppert M (1990) Mapping recessive ophthalmic diseases: Linkage for Usher syndrome type II to a DNA marker on chromosome 1q. Genomics *7:*250–256.

Mindrinos M, Katagiri F, Yu GL, and Ausubel M (1994) The A. thaliana disease resistance gene RDS2 encodes a protein containing a nucleotide-binding site and leucine-rich repeats. Cell *78:*1089–1099.

Ollendorff V, Szepetowski P, Mattei MG, Gaudray P, and Birnbaum D (1992) New gene in the homologous human 11q13-q14 and mouse 7F chromosomal regions. Mammalian Genome *2:*195–200.

Ollendorff V, Noguchi T, deLapeyriere O, and Birnbaum D (1994) The GARP gene encodes a new member of the family of leucine-rich repeat-containing proteins. Cell Growth Differentation *5:*213–219.

Roberts DF and Bonné B (1973) Reproduction and inbreeding among the Samaritans. Soc. Biol. *20:*64–70.

Sambrook J, Fritsch EF, and Maniatis T (1989) Molecular Cloning: A Laboratory Manual. Second edition. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Sankila EM, Pakarinen L, Kaariainen H, Aittomaki K, Karjalainen S, Sistonen P, and de la Chapelle A (1995) Assignment of an Usher syndrome type III (USH3) gene to chromosome 3q. Hum. Mol. Genet. *4:*93–98.

Seroussi E, Korostishevsky M, Sheffield VC, Gerhard D, and Bonné-Tamir B (1994) Haplotype analysis and physical mapping to determine the position of Usher syndrome type I (USHIB) among closely linked microsatellites on chromosome 11q13.5. The 4th Chromosome 11 Workshop. Oxford, UK. Abstract.

Smith RJH, Lee EC, Kimberling WJ, Daiger SP, Pelias MZ, Keats BJB, Jay M, Bird A, Reardon W, Guest M, Ayyagari R, and Hejtmancik JF (1992) Localization of two genes for Usher syndrome type I to chromosome 11. Genomics *14:*995–1002.

Weil D, Blanchard S, Kaplan J, Guilford P, Gibson F, Waish J, Mburv P, Varela A, Levilliers J, Weston MD, Kelly PM, Kimberling WJ, Wagenaar M, Levi-Acobas F, Larget-Piet D, Munnich A, Steel KP, Brown SDM, and Petit C (1955) Defective Myosin VIIA gene responsible for Usher syndrome type IB. Nature *374:*60–61.

Whitman S, Dinesh-Kumra SP, Choi D, Hehl R, Corr C, and Baker B (1994) The product of the tobacco mosaic virus resistance gene N: Similarity to toll and the interleukin-1 receptor. Cell *78:*1101–1115.